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J. Helminthol. Soc. Wash.
61(2), 1994, pp. 249–252

Research Note

Identification of a *Haemonchus placei*-Specific DNA Probe

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ABSTRACT: A partial DNA library was generated from *Haemonchus placei* and differentially screened to identify clones containing repetitive and species-specific sequences. A DNA sequence, which hybridized with *H. placei* genomic DNA by dot-blot analysis and did not hybridize with *Haemonchus contortus* DNA, was identified and characterized. The probe, designated pHp3, was sequenced and found to be 723 bp in length, constituting 0.34% of the *H. placei* genome. The pHp3 probe is useful in differentiating the morphologically similar parasites *H. placei* and *H. contortus.*

KEY WORDS: *Haemonchus placei, H. contortus,* DNA probe, species specific.

Haemonchus is a genus among trichostrongyle nematodes that parasitizes the abomasum of ruminants. The different *Haemonchus* species (9–10 species have been recognized [Gibbons, 1979; Lichtenfels et al., 1993]) develop in a variety of domesticated and wild ruminant hosts, with *Haemonchus placei* and *Haemonchus contortus* being species typically found in cattle and sheep, respectively (see Lichtenfels et al., 1986). Another species, *H. similis,* occurs in cattle especially in southern North America, Central and South America, but it is easily distinguished morphologically from *H. contortus* and *H. placei.* Mixed infections with the 2 morphologically similar species, *H. placei* and *H. contortus,* occur in both domesticated hosts, leading to discussion of the validity of 2 species. Some authors (Gibbons, 1979) have synonymized *H. contortus* and *H. placei* while others (Le Jambre, 1979, 1981; Lichtenfels et al., 1986, 1993) have provided evidence for the recognition of both species. Lichtenfels et al. (1986, 1988, 1993) have described morphological characteristics in detail for identification of individual worms of the 2 species, i.e., cuticular ridge patterns and spicule lengths. These characters make it difficult to differentiate female worms without training and require the recovery of adult worms and hence killing of the host.

In the current report, we describe the development and application of an *H. placei*-specific DNA probe, which can discern *H. placei* and *H.*

Nucleotide data reported in this paper have been submitted to the Genbank™ data base with the accession number: L20568.

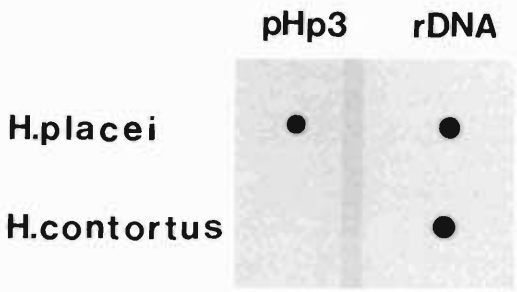

Figure 1. Dot-blot analysis demonstrating genus specificity of cloned repetitive sequence, pHp3. Genomic DNA (~0.2 μg) of *H. placei* and *H. contortus* was heat denatured, vacuum filtered onto Nytran^R membranes, and screened with radiolabeled probes (pHp3; rDNA). The probes were hybridized overnight to the blots at 65°C in hybridization solution (0.6 M NaCl, 0.5% SDS, 1% Denhardt's solution (80×), 10 μg/ml salmon sperm DNA, 10 μg/ml heparin) then washed (2 × 30 min) in 0.1% SDS and 0.2× SSC (1× SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) at 60°C.

contortus, and which may additionally be adapted to an antemortem test to differentiate *Haemonchus* eggs excreted in the feces, thereby identifying the presence of *H. placei* in infected animals.

The pHp3 probe was developed using methods described by Christensen et al. (1994). Differential screening of a *H. placei* genomic DNA library, using radiolabeled *H. placei* and *H. contortus* genomic DNA, was performed to identify clones containing repetitive and species-specific sequences. Reactive clones were selected and rescreened with radiolabeled homologous genomic DNA to select the clones giving the strongest reactions.

To verify the specificity of pHp3 for *H. placei*, equivalent amounts (approximately 0.2 μg) of genomic DNA from *H. placei* and *H. contortus* were heat-denatured and blotted onto duplicate Nytran^R membranes. The blots were screened

pHp3

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1    TATGTCCTGT AGAGGAGAAA ATACCATAAA ATCGATGCTC CTGAACGAAG GTGTTTCAGA
61   GAATGAGAAA AGCTGAGTGG AAACTAGCAC AGAAAGCGAG ATCCCGTTCC TAGCTCCAAA
121  TAGTGCATGG CTCATTCGTA GACGGTCTTG GGGAAGCCAT CGAAATGGTA CAGGGCTTCC
181  GATACCTGAA AAGTACTTGC CGATGATGGC AGCGTGGACC AGGCGGTAAA AGCCAGAGTA
241  ATCGAGGCGT GGATGAGGTG GAGGGAATTC ACCGTTATCC TCTACGACCA TAGGTGCTTA
301  GGAGTCAGAG GAAAGGGATA CCAAACAGTA GTTAGGCCCA TGCAGTGGCT GGCAGCCGAG
361  ATGGTGCTAG GAGGAGGGAG GGAGACAGAC AGGACCCTTC TACGCGGTCG CGTCGAGCCC
421  ATGTCCCTTC GAAGCTGAAA ACAAGGAGAA ACAAGATCAG AACAATTTGC CTTTGCCCTT
481  TTTCCCTTGG GCGCGACGCG ACCGCGTTGA AAGGTCCCGT CTGCCTCCTC TCCCACCCAG
541  CACTACCTCG GCGGCACGCC GGCACGCCGG TCGGCCGGAC TATGAAAGCA GCCAGTGCTT
601  TACGGCAGCG AATGTTGGCC GTTGGTAAAC ACATGAAAGA CAACTGCACT CTGCTGAAAT
661  GAGGATGTTG CGGTTGGAAG CGACTGGATG GGATCCGTGA AGTGCTACGA GAACTAATTT
721  GAG
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Figure 2. Nucleotide sequence of *H. placei*-specific repetitive sequence, pHp3.

with radiolabeled probes (Feinberg and Vogelstein, 1983) of either pHp3 DNA or ribosomal DNA (rDNA) (Zarlenga et al., 1994). The blots were washed under high stringency conditions (60°C, 0.2× SSC, 0.1% SDS) to reduce nonspecific hybridizations. The pHp3 probe reacted only with *H. placei* genomic DNA, whereas the rDNA probe hybridized equally to genomic DNA from both *H. placei* and *H. contortus,* thus verifying the presence of equivalent amounts of genomic DNA on the filter (Fig. 1). The specificity of the pHp3 probe was tested and confirmed using *H. placei* and *H. contortus* laboratory strains maintained at Beltsville, Maryland, and 3 different *H. contortus* strains provided by G. Conder at The Upjohn Company. Previous results (Zarlenga et al., unpubl. obs.) demonstrated that these parasite species (wild type and domestic type) from various geographic locations are genetically similar. Thus, we suggest that this probe will have unilateral application in differentiating *H. placei* and *H. contortus.*

The nucleotide sequence of pHp3 was determined by dideoxy sequencing (Sanger et al., 1977) using the Sequenase™ kit (US Biochemical Corp., Cleveland, Ohio) in both directions employing both forward and reverse *pUC* primers as well as synthetic internal primers. The cloned sequence was 723 bp in length, containing 47% AT, with no notable internal repeats (Fig. 2). The clone constitutes approximately 0.34% of the total genome (data not shown), determined as previously described (Zarlenga et al., 1991).

Techniques similar to those cited above are commonly applied to identify sequences repeated within the parasite genome (Zarlenga et al., 1991; Egwang et al., 1992; Christensen et al., 1994). Such repeats are believed to represent noncoding regions of the genome, which tend to undergo evolutionary change at a faster rate than coding regions. This makes these regions excellent targets for the identification of species-specific sequences (Flavell, 1982; Hammond and Bianco, 1992).

The application of the described probe requires only small amounts of DNA. In addition, radiolabeled pHp3 can be used to identify DNA extracted from *H. placei* eggs (extracted as described by Christensen et al. [1994]). With this probe the presence of *H. placei* in animals can be determined. As such this probe will be very useful in defining the distribution of *H. placei* in cattle herds without killing of selected individuals, and as a research tool to verify the purity of *H. contortus* strains. Complete definition of the distribution of *H. placei* and *H. contortus* will require the identification of a probe specific for both *H. contortus* and *H. placei* as the use of the present probe cannot rule out the presence of *H. contortus* in infected animals.

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Interest received in 1993	$884.64
Donations, including a memorial for Frank D. Enzie	$1,025.00
Total	$1,909.64
Disbursements:	
Grant to the Helminthological Society of Washington for 1993	($50.00)
Membership in the American Association for Zoological Nomenclature for 1993	($50.00)
Page Chart Support	($180.00)
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Balance on hand, December 31, 1993	$14,773.12

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